PHYSIOLOGY

Photoreceptive Features of Vesicles associated with the Nervous System of Cephalopods

FROM the results of an investigation using both the light and the electron microscope Nishioka, Hagadorn and Bern[1] concluded that the epistellar body of *Octopus bimaculatus*(?) found off the coast of California was probably a photoreceptive organ. Present within the lumen of the epistellar body are processes equipped with microvilli; these are often organized into rhabdomes as in the eyes of many protostome invertebrates[2]. In this species of *Octopus*, as well as in others studied, the epistellar body is only barely discernible as a pale orange spot at the base of the posterior stellar nerves of the stellate ganglion. In *Eledone*, however, the similarly located epistellar body is much larger and more intensely orange than in *Octopus*. Even with light microscopy the reticular pattern suggestive of rhabdomeric organization was apparent at the periphery of the lumen. The morphology indicative of a photoreceptive function and the large amount of pigment observed provided the basis for the present investigation of the biochemical nature of the pigment of the epistellar body of *Eledone moschata*. The details of this study and related information on vesicles associated with the nervous system of cephalopod molluscs will be reported elsewhere[3].

Sixty-three *E. moschata* were dark-adapted for at least 16 h before killing them. The stellate ganglia and eyes were then removed under red light. Both tissues were immediately frozen in beakers suspended over dry ice in acetone. After thawing and further dissection, the portion of the stellate ganglia which contained the epistellar bodies and the remainder of the stellate ganglia were homogenized separately and centrifuged in a sucrose gradient. The eyes were also homogenized and centrifuged in a similar sucrose gradient after an initial crude separation of cellular material from pigment granules. Material floating in the 0·5 M sucrose concentration was then removed and mixed with 1 M disodium acid phosphate and centrifuged. The precipitate was dissolved in digitonin and transferred to a Beckman cuvette, and the absorption spectrum characteristic of rhodopsin was obtained on the eye material using a Beckman ratio recording spectrophotometer. A similar spectrum was obtained with material from the epistellar body. The pH of the solution was then raised slightly above 9·0 with sodium carbonate, and the solutions containing rhodopsin were exposed to room light for about 1 h before a second absorption spectrum was obtained; this recorded the shift to metarhodopsin in both instances (Table 1). The λ_{max} of rhodopsin in the eye[4] was slightly higher than in the epistellar body, whereas the λ_{max} of alkaline metarhodopsin was identical for both organs. These curves were obtained on three separate batches of material, and the data for one run are given in Table 1. No evidence for the presence of rhodopsin–metarhodopsin was obtained from the stellate ganglion (control tissue preparations).

In their earlier paper, Nishioka *et al.*[1] concluded that the epistellar body could not be a neuroendocrine structure[5-7] or the homologue of the third-order giant fibre system of squids[5]. We have suggested that the parolfactory vesicles in decapod cephalopods[7] were more likely homologues of the octopod epistellar body. Accordingly, the parolfactory vesicles of the squid *Loligo vulgaris* were also investigated. Histological examination of the optic tract of decapod molluscs has shown the presence of a number of vesicular structures containing processes similar to those of the octopod epistellar body[7]. These vesicles vary in number from four to fifteen on each side and lie superficially in connective tissue adjacent to the olfactory lobes—thus the name "parolfactory". Because of their minute size, we initially expected that they would be extremely difficult to find in fresh material; however, here again the intense orange pigmentation facilitated their collection.

Parolfactory vesicles from ninety-two *Loligo vulgaris* were used. Except for a shorter duration of dark adaptation (about 6 h)—because of the higher mortality of squids in captivity—the experimental procedure was identical with that used on *Eledone* material. Pieces of optic lobe approximately equal in volume to the parolfactory vesicles in their enveloping connective tissue were taken as control tissue, and the eyes[8] were again used for comparison. The λ_{max} of rhodopsin and of metarhodopsin from one of two determinations are given in Table 1. The optic lobes showed no evidence of visual pigment.

To substantiate further the specificity of the biochemical features of these photoreceptive structures, the antimony chloride method for determination of vitamin A_1 (see refs. 8 and 9) was used on extracts. Vitamin A_1 (ref. 9) was detected in both organs, but none was found in the control tissues (the remainder of the stellate ganglion in the octopus and parts of the optic lobe in the squid).

Because of the small size of both epistellar and parolfactory structures, no weights were taken. Nevertheless, we are impressed by the appreciable amounts of visual pigment isolated from these organs. Gross comparison with the eye leads us to believe that the concentration of visual pigment in the epistellar body and parolfactory vesicles is probably greater than in the eye.

We conclude from the biochemical information presented here that the epistellar body and the parolfactory vesicles contain considerable amounts of visual pigments very similar to those present in the eyes of the same animals. Our initial belief that the epistellar body was a vestigial organ in the adult organism[1] (conceivably important only in larval life) may now be untenable. It seems more likely that these organs are of importance in photoperiodic regulation in the adult, despite their cryptic locations. The vesicles are not to be viewed as "3rd and 4th eyes"[10], inasmuch as they would scarcely seem responsible for vision (that is to say, image perception). Photoreceptive function can extend to physiological regulations and include much more than vision *per se*. It is to this more general aspect that comparative physiologists need now address themselves.

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Table 1. VISUAL PIGMENTS IN CEPHALOPOD TISSUES

Tissue	No. of animals	λ_{max} rhodopsin	λ_{max} metarhodopsin
Eledone eye	4	475	380
Eledone epistellar body	20	470	380
Loligo eye	2·5	493	380
Loligo parolfactory vesicles	57	495	380